



TEST REPORT

Analysis of the Sanitation Performance of LaundryPure

Client: EcoQuest International
Client Number : 3P300
NSF Program: 540
Product: LaundryPure
Serial Number: LAP120 107355
Job Number: J-00027764
Date of Experimentation: June 3, 2006 through June 7, 2006
Overall Study Result: COMPLETE

Study Director: _____ **Date:** _____

QC Review: _____ **Date:** _____



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STUDY PERSONNEL

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METHODOLOGY

Inoculum Preparation

The culture preparation and transfers were conducted individually and separately for each organism. The cultures of *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538, and *Klebsiella pneumoniae* ATCC 4352 obtained from ATCC were resuspended in individual 10 ml of Tryptic Soy Broth (TSB) and incubated for 48 hours at 35±1°C. One mL of the 24-hour suspension was transferred to 10 mL of fresh TSB and incubated for 24 hours at 35±1°C. This passing was repeated for a minimum of 3 consecutive daily transfers (no greater than 30 transfers). On the day before testing, the entire 10 mL of the organism stock was transferred into a 2 L sterile Erlenmeyer flask containing 1 L of fresh TSB. The flask was placed on a rotary shaker set at 150 rpm. The test suspension was incubated while shaking for 24 hours at 35±1°C. After the 24-hour incubation, the 1 L culture was divided into 250 mL aliquots and placed into sterile centrifuge tubes. The suspension was centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted and the pellet resuspended in 25 mL of sterile buffered deionized water (SBDW). The entire centrifugation and washing process was repeated for a total of three times. After the third centrifugation event, the pellet was resuspended in 75 mL of SBDW. This typically yielded a target concentration between 10⁹ and 10¹⁰ CFU/mL (Colony Forming Units per milliliter) for each test organism. Density determination was carried out through serial tenfold dilutions of the suspensions into SBDW. The resulting dilutions were plated in triplicate using the following growth media: Violet Red Bile Agar (VRBA) for *K. pneumoniae*, Pseudomonas Isolation Agar (PIA) for *P. aeruginosa*, and Mannitol Salt Agar (MSA) for *S. aureus*.

Pre-conditioning of Washing Machine

Per the instruction manual, the washing machine was pre-conditioned prior to using the LaundryPure unit to remove any detergent buildup. The machine was run four times utilizing the large load size and cold water without laundry added.

Laundry Load

An eight pound AHAM Standard Mixed Load, as specified in Table 1 of Performance Evaluation Procedure for Household Washers (ANSI/AHAM HLW-1), was used for testing, representing a relatively heavy clothes



loading. Preconditioned AHAM Standard Mixed Loads were received from Textile Innovators Corporation. This Test Load contained the following number and types of items:

2 sheets	3 shorts	1 tablecloth	2 pillow-cases
2 shirts	2 handkerchiefs	3 bath towels	1 washcloth
2 tee shirts			

Soiling

To simulate typical soil loading into the machine, the following Soiled Swatches were added to the clothing load, representing approximately 20 grams of soil:

- Five 3 x 3.5 inch (7.62 x 8.89 cm) swatches with 1 gram of dust sebum (providing oil and grease);
- Five 3 x 3.5 inch (7.62 x 8.89 cm) swatches with 1 gram of ground in clay per swatch (providing particulates);
- Five 3 x 3.5 inch (7.62 x 8.89 cm) swatches with 1 gram of beef gravy per swatch; and
- Five 3 x 3.5 inch (7.62 x 8.89 cm) swatches with 1 gram of cocoa/milk/sugar.

Soiled Swatches were received from Scientific Services.

Sanitization Testing

The LaundryPure unit’s antimicrobial efficacy was initially evaluated against *K. pneumoniae* ATCC 4352. The efficacy versus *S. aureus* ATCC 6538 and *P. aeruginosa* ATCC 15442 was evaluated following completion of the *K. pneumoniae* study.

On each challenge day, the LaundryPure unit was connected to a Kenmore 70 Series Washer and evaluated on the cold/cold cycle using the heavy duty setting on large load size (30 minute estimated run time). No detergent was used in testing. A pre-washed, sterile, eight pound AHAM Standard Mixed Cotton Test Load, as specified in Table 1 of ANSI/AHAM HDL-1, was aseptically transferred to the washer. Three 12 x 12 inch pieces of heavy blue denim material, which were hemmed by folding up ½ inch on each edge and sewn to prevent fraying, were inoculated with 2 mL of the challenge organisms to achieve the target minimum inoculation concentration of 1 x 10⁹ CFU/mL. Following completion of the test cycle, the denim swatches were transferred to a neutralizer / stomaching solution consisting of phosphate buffered water amended with 1% Tween 80, lecithin and Chamber’s solution (for silver neutralization). The eluent solution was analyzed for challenge organism concentration via spread plating on the following selective media: Baird Parker Agar was used for *S. aureus*; Pseudomonas



Isolation Agar was utilized for *P. aeruginosa*; Violet Red Bile Agar was used for the enumeration of *K. pneumoniae*. Three positive control swatches were used for each test run. The positive control swatches were inoculated with the challenge organisms in the same manner described previously. However, the positive control swatches were not exposed to the washing cycle. The positive control swatches were stomached and plated within 15 minutes of inoculation.

To evaluate the potential carryover of challenge organisms between cycles, a sterile eight pound AHAM Standard Mixed Cotton Test Load, as specified in Table 1 of ANSI/AHAM HDL-1 was aseptically transferred to the washer following a challenge run concurrently with three uninoculated, sterile, 12 x 12 inch, heavy weight blue denim swatches (negative control). The washer was disconnected from the LaundryPure unit and set to the same cycle settings and run time as the test runs. Following the run, the negative control swatches were assayed for the presence of the challenge organisms as described previously.

All positive control, challenge runs and negative control test runs were repeated in triplicate for each challenge organism. All testing was performed at NSF laboratories in a room maintained at $75 \pm 5^\circ\text{F}$ and $50 \pm 10\%$ relative humidity. The installation of the LaundryPure unit was performed according to the manufacturer's instruction manual.

Data Analysis

For each individual test run, the following calculations were performed:

$$\text{Percent reduction} = (\text{ACC} - \text{ATC}) / \text{ACC} \times 100$$

$$\text{Log Reduction} = \log (\text{ACC} / \text{ATC})$$

Where: ACC = average Positive Control Sample (swatch) organism count before cycle (CFU/mL)

ATC = average Test Sample (swatch) organism count after cycle (CFU/mL)



RESULTS

The data obtained from the challenge studies is presented in Appendix A. The minimum target cell concentration was achieved for each challenge organism in each test run. The Client's product achieved greater than a 6-log reduction (>99.9999% reduction) for *S. aureus* ATCC 6538 and *K. pneumoniae* ATCC 4352. *P. aeruginosa* ATCC 15442 demonstrated between 1.79 and 1.93 log reduction (98.38 and 98.79% reduction). No challenge organisms were detected on any of the negative control swatch samples.



APPENDIX A

TABLES AND FIGURES



Table 1. Percent reduction and log reduction values for the triplicate challenge runs for *S. aureus* ATCC 6538.

Challenge Run	Percent Reduction	Log Reduction
Run 1	>99.9999	>6.24
Run 2	>99.9999	>6.35
Run 3	>99.9999	>6.11

Table 2. Results for Negative Control runs for *S. aureus* ATCC 6538. Challenge organism counts are presented in CFU/L. The limit of detection is 10 CFU/L and is the average of three replicates.

Negative Control Run	Concentration of Challenge Organism
Run 1	<10
Run 2	<10
Run 3	<10



Table 3. Percent reduction and log reduction values for the triplicate challenge runs for *P. aeruginosa* ATCC 15442.

Challenge Run	Percent Reduction	Log Reduction
Run 1	98.79	1.92
Run 2	98.38	1.79
Run 3	98.84	1.93

Table 4. Results for Negative Control runs for *P. aeruginosa* ATCC 15442. Challenge organism counts are presented in CFU/L. The limit of detection is 10 CFU/L and is the average of three replicates.

Negative Control Run	Concentration of Challenge Organism
Run 1	<10
Run 2	<10
Run 3	<10



Table 5. Percent reduction and log reduction values for the triplicate challenge runs for *K. pneumoniae* ATCC 4352.

Challenge Run	Percent Reduction	Log Reduction
Run 1	>99.9999	>6.34
Run 2	>99.9999	>6.38
Run 3	>99.9999	>6.11

Table 6. Results for Negative Control runs for *K. pneumoniae* ATCC 4352. Challenge organism counts are presented in CFU/L. The limit of detection is 10 CFU/L and is the average of three replicates.

Negative Control Run	Concentration of Challenge Organism
Run 1	<10
Run 2	<10
Run 3	<10



REFERENCES

ANSI/AHAM HLW-1, "Performance Evaluation Procedure for Household Washers",
Association of Home Appliance Manufacturers, Chicago, IL, USA.

AOAC Official Methods of Analysis, Volume 1, Disinfectants Section 960.06D.
"Preparation of Culture Suspension".

Standard Methods for the Examination of Water and Wastewater, 20th edition.